



Evaluation of the effect of ionic liquids as adjuvants in polymer-based aqueous biphasic systems using biomolecules as molecular probes



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ABSTRACT

Aqueous biphasic systems (ABS) have been largely investigated for the extraction, separation and/or purification of biomolecules. Recently, the use of ionic liquids (ILs) as additives in conventional polymer-based ABS was proposed to overcome the limited range of polarities of the coexisting phases. However, the impact of ILs on the partitioning of biomolecules on IL additivated ABS is not universal and is still poorly understood. Aiming at obtaining additional insights on this matter, the effects of the chemical structure of the IL, tie-line length (TLL) and biomolecule nature upon the partition of a series of model biomolecules were investigated. For this purpose, ternary ABS (composed of polyethylene glycol (PEG) 400, citrate buffer at pH 7.0, and water), and several quaternary ABS (composed of PEG 400, citrate buffer at pH 7.0, water and ILs at 5 wt%), were prepared using different chloride-based ILs ([C₄mim]Cl, [C₄mpyr]Cl, [C₄mpip]Cl, [P₄₄₄₄]Cl and [N₄₄₄₄]Cl). The partition of a wide range of biomolecules in these systems (gallic acid, vanillic acid, eugenol, nicotine, caffeine, L-tryptophan, L-phenylalanine and L-tyrosine), used here as molecular probes, was studied. These solutes were chosen due to their wide range of polarities. The results obtained support the concept that ILs, when used as adjuvants in polymer-based ABS, change the coexisting phases' characteristics and modify the partition behavior of biomolecules. In general, a positive effect derived from the use of ILs as adjuvants in PEG-salt systems is observed, particularly when dealing with more hydrophobic biomolecules, whereas IL + salt ABS perform better in the extraction of more hydrophilic biomolecules. The favourable partition of more hydrophilic biomolecules in IL + salt ABS seems to be ruled by specific interactions with the IL, while the favourable partition of more hydrophobic biomolecules in PEG + salt and PEG + salt + IL seems to be governed by the differences in the phases hydrophobicities. It is shown that ILs preferentially migrate to the PEG-rich phase, and that there is a correlation between the partition coefficients of the biomolecules and ILs and the biomolecules octanol-water partition coefficients.

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1. Introduction

Most processes used for biomolecules extraction and purification are generally expensive as they require multiple, often non-conventional, unit operations. In addition, many processes require long extraction times, have low efficiency, and some lead to a decrease on the biological activity of the target biomolecules [1–4]. There has been, therefore, a strong interest on the development of alternative separation and purification processes for biomolecules [3,5–7]. Among the various processes studied, liquid-liquid extraction (LLE) is known to have several advantages, such as high yields and selectivity, technological simplicity, and low cost. How-

ever, LLE is usually carried out with organic solvents, which may have a negative impact on the biomolecules, environment and human health. For these reasons, aqueous biphasic systems (ABS) have been studied as an alternative to conventional organic/aqueous systems on LLE processes [2,8–10].

Typical ABS consist on two macroscopic liquid phases that coexist in equilibrium and are formed by the dissolution in water, at appropriate concentrations, of two polymers, a polymer and a salt, or two salts [2,8,9,11–13]. Their advantages comprise a low interfacial tension, fast phases separation, low cost, easy scale-up, and high water content, providing favourable conditions to the design of biomolecules extraction/separation processes. The biomolecules partition between the ABS phases depends upon their affinity for each of them, which further depends on parameters such as pH, temperature and system composition [2,8,10,14].

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Polyethylene glycols (PEGs) are polymers widely used in the preparation of ABS due to their biocompatibility, biodegradability, low toxicity and low-cost [15]. However, polymer-based ABS exhibit a restricted difference in polarities between the two phases which limits their application for separation purposes [16–19]. Pereira et al. [18] suggested that ionic liquids (ILs) could be applied in conventional ABS, in small amounts, to overcome this limitation. ILs are low melting temperature organic salts, being also non-volatile, non-flammable, and with high chemical and thermal stabilities [20]. Moreover, they display a high solvation ability for a wide range of compounds, that it is further possible to manipulate by an adequate combination of different cations and anions [20–22]. Pereira et al. [18] evaluated the partitioning of L-Tryptophan in ABS composed of PEG 600 (40 wt%) and Na₂SO₄ (5 wt%), with the addition of 5 wt% of imidazolium-based ILs as adjuvants. The authors [18] observed that the addition of ILs as adjuvants modifies the phases characteristics and the extraction capability of the ABS. The partition coefficients were shown to be mainly dependent on the IL cation alkyl side chain length, where ILs with shorter and longer alkyl chains provide lower and higher values of partition coefficients than in ABS without IL, respectively. Other IL concentrations (5–10 wt%) were further investigated by Almeida et al. [22] in ABS constituted by PEG 300 (23 wt%) and Na₂SO₄ (12 wt%) for the extraction of gallic, vanillic and syringic acids. The results obtained demonstrated the ability of ILs to tune the polarity of the PEG-rich phase [22]. The antioxidants partition coefficients were shown to depend on the PEG molecular weight and IL employed, with the use of 5 wt% of IL providing enhanced extraction efficiencies in comparison to ABS without IL. Overall, these results indicated that the chemical nature of the IL controls the preferential partition of the biomolecules for a given phase [22]. However, not all results concerning the use of ILs as adjuvants in ABS were equally successful, i.e., provided higher partition coefficients or extraction efficiencies of the IL-containing systems for biomolecules when compared to the systems without IL. Souza et al. [19] studied the partition of Chloranilic Acid and Rhodamine 6G in ABS composed of PEG 1500 or 8000 (15 wt%), phosphate buffer at pH 7.0 (15 wt%) and 5 wt% of the several chloride-based ILs. The authors [19] found that the addition of ILs to PEG-salt ABS does not increase the partition of dyes to the PEG-rich phase in comparison to the ABS without IL. The same researchers [7] attempted the use of imidazolium-based ILs as adjuvants (5 wt%) in ABS composed of PEG (1500, 4000, 6000 and 8000) (15 wt%) and potassium phosphate buffer at pH 7 (15 wt%) for the extraction of a lipase produced by submerged fermentation by *Bacillus* sp. ITP-001. Although they achieved higher purification factors for the enzyme in quaternary systems containing ILs as adjuvants than in ternary ABS (PEG + Salt + water), this trend was only observed when using ILs with long cation alkyl side chains [7]. Ferreira et al. [23] used ABS composed of PEG 400 (25 wt%), potassium citrate buffer at pH 7.0 (25 wt%), and ILs as adjuvants, to extract/purify Immunoglobulin G (IgG) from a rabbit serum source. While an increase in the extraction efficiency of IgG from 96% (without IL) to 100% was observed for the systems containing 5 wt% of some ILs, the ABS containing bromide-based ILs led to a decrease on the recovery yield of the target protein. Overall, it is clear that the use of ILs as adjuvants to enhance the performance of polymer-based ABS is not an universal solution, further requiring additional studies for a better understanding of these systems.

There is still a poor understanding on polymer-based ABS when employing ILs as adjuvants, and we lack today the fundamental knowledge required to develop and optimize separation procedures based on these systems. This work aims at overcoming these limitations by focusing on the study of several quaternary ABS, based in polyethylene glycol (PEG 400), potassium citrate/citric acid buffer and several chloride-based ILs as adjuvants at 5 wt%.

The citrate salt was chosen because it is biodegradable, nontoxic, and allows to work in a fixed pH when used as a buffer combined with citric acid. A fixed pH is a quite relevant parameter regarding the objectives of the current work given that all biomolecules evaluated can suffer pH-driven speciation. All liquid–liquid systems were evaluated regarding their extraction potential for seven biomolecules, including phenolic antioxidants (vanillic acid, gallic acid and eugenol), alkaloids (nicotine and caffeine) and amino acids (L-Tryptophan, L-Phenylalanine and L-Tyrosine), here used as molecular probes for a better understanding of solutes partitioning in polymer-salt ABS using ILs as adjuvants. The partition coefficients obtained for these quaternary ABS were compared to those obtained for ternary ABS composed of PEG 400 and potassium citrate buffer (without ILs) or composed of ILs and potassium citrate buffer (without PEG).

2. Experimental section

2.1. Materials

The ABS studied in this work are constituted by water, potassium citrate/citric acid buffer at pH 7 (wt%), polyethylene glycol with a molecular weight of 400 g mol⁻¹ (PEG 400) and several chloride-based ILs. PEG 400 was supplied by Sigma-Aldrich. Tri-potassium citrate monohydrate (99 wt% pure) and citric acid (99 wt% pure) were purchased from Prolabo and Fisher Scientific, respectively. 12996545123825 The water employed was double distilled, passed across a reverse osmosis system and finally treated with a Milli-Q plus 185 water purification apparatus. To these systems, 5 wt% of several ILs were added. The ILs studied were: 1-butyl-3-methylimidazolium chloride, [C₄mim]Cl (>99% pure); 1-butyl-1-methylpyrrolidinium chloride, [C₄mpyr]Cl (>99% pure); 1-butyl-1-methylpiperidinium chloride, [C₄mpip]Cl (>99% pure); tetrabutylammonium chloride, [N₄₄₄₄]Cl (>97 wt% pure); and tetrabutylphosphonium chloride [P₄₄₄₄]Cl (>96% pure). The [C₄mim]Cl, [C₄mpyr]Cl and [C₄mpip]Cl were purchased from Iolitec, while [N₄₄₄₄]Cl was acquired from Sigma-Aldrich and [P₄₄₄₄]Cl was kindly supplied by Cytec Ind. The chemical structures of the ILs studied are shown in Fig. 1.

The antioxidants vanillic acid (97 wt% pure) and eugenol (99 wt% pure) were acquired from Sigma-Aldrich, and gallic acid (99.5 wt% pure) from Merck. The alkaloids nicotine (99 wt% pure) and caffeine (99 wt% pure) were supplied by Fluka. The amino acids L-Tryptophan (99 wt% pure) and L-Phenylalanine (99 wt% pure) were acquired from Sigma-Aldrich, and L-Tyrosine (99 wt% pure) from Fluka. The molecular structures of the biomolecules investigated and their characteristics, such as their molecular weight (*M_w*), octanol-water partition coefficient (*K_{ow}*) and solubility in water, are provided in Table 1.

2.2. Biomolecules partition in ABS

The phase diagrams for the ABS here studied were reported elsewhere by Sousa et al. [25]. Aiming at a better understanding of the characteristics of polymer-salt-based ABS when ILs are used as adjuvants, the partition coefficients of the several biomolecules investigated were also determined and compared with those obtained in ternary ABS composed of polymer and salt (without IL) or of ILs and salt (without PEG). The following mixture compositions were initially used: 30 wt% of PEG + 30 wt% of salt or 30 wt% of IL + 30 wt% of salt for ternary ABS; and 30 wt% of PEG + 30 wt% of salt + 5 wt% of IL for quaternary ABS. This mixture point was chosen with the objective of keeping a similar volume of the phases thus minimizing the effect of the volume ratio. These fixed compositions also allow to evaluate the partition coefficients of each

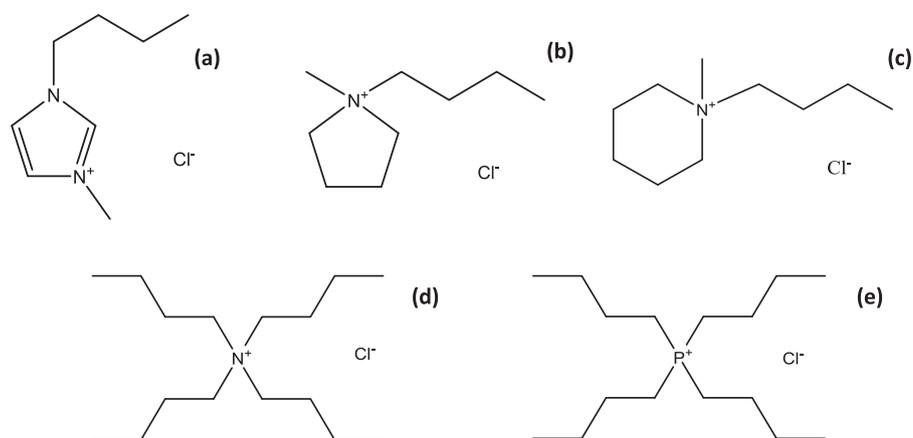


Fig. 1. Chemical structures of the studied ILs: (a) [C₄mim]Cl, (b) [C₄mpyr]Cl, (c) [C₄mpip]Cl, (d) [N₄₄₄₄]Cl and (e) [P₄₄₄₄]Cl.

Table 1
Molecular structures and characteristics of the studied biomolecules [24].

Name	Chemical structure	log <i>K</i> _{ow}	<i>M</i> _w /(g mol ⁻¹)	Solubility in water at 298 K/(g L ⁻¹)
Gallic acid		0.70	170.12	11.9
Vanillic acid		1.33	168.14	1.5
Eugenol		2.49	164.20	2.46
Caffeine		-0.07	194.19	21.6
Nicotine		1.17	162.23	Miscible
L-Tryptophan		-1.06	204.23	13.4
L-Phenylalanine		-1.38	165.19	25.9
L-Tyrosine		-2.26	181.17	0.48

biomolecule as a function of the tie-line length (TLL). The TLL of each mixture compositions was determined by a gravimetric approach taking into account the fitting of the binodal curve and weight of each phase [18], and are presented in the Supporting Information (Table S1).

Aiming at avoiding the interference of TLL values in the partition coefficients, new experiments were carried at a constant TLL (ca. 70). These composition points are reported in Table S2 in the Supporting Information. Aqueous solutions of each biomolecule

(0.5 g L⁻¹ for gallic acid and vanillic acid, 0.1 g L⁻¹ for eugenol and L-Tyrosine, 1.0 g L⁻¹ for caffeine, nicotine and L-Tryptophan, and 3.0 g L⁻¹ for L-Phenylalanine) were used as part of the water composition. All phase-forming components were weighted within ±10⁻⁵ g, vigorously stirred for 5 min, and allowed to equilibrate at 298 (±1) K during 12 h. After this period, the top and bottom phases were carefully separated and weighted. The biomolecules concentration in each phase was measured by UV-spectroscopy, using a SHIMADZU UV-1700, Pharma-Spec Spectrometer, at a

wavelength of 262 nm for gallic acid, 292 nm for vanillic acid, 279 nm for eugenol, 273 nm for caffeine, 260 nm for nicotine, 279 nm for L-Tryptophan, 275 nm for L-Tyrosine, and 258 nm for L-Phenylalanine, using calibration curves previously established. Aiming at avoiding the interference of the salt, PEG and IL in the quantification, blank control samples were always used. At least two individual experiments were carried out for each ABS, allowing the determination of the average partition coefficient and respective standard deviations.

The partition coefficients of each biomolecule were determined according to Eq. (1),

$$K = \frac{C_T}{C_B} \quad (1)$$

where C_T and C_B are the concentrations (g L^{-1}) of the biomolecule in the top and in the bottom phase, respectively. It should be remarked that the top phase corresponds to the PEG-rich phase in both the polymer-salt and polymer-salt ABS with ILs as adjuvants, and to the IL-rich phase in the IL-salt ABS.

2.3. ILs partition in ABS

For the ABS composed of PEG 400 and the citrate-based buffer containing the IL at a fixed concentration (5 wt%), it was also determined the partition coefficient of the IL for a better understanding of the biomolecules partition. Partition coefficients for the IL were determined at a constant TLL (*ca.* 70), also used in the extraction experiments using biomolecules. The IL concentration in each phase was quantified using a Metrohm 904 Titrand ion chloride electrode. A stock aqueous solution of KCl (1 mol L^{-1}) was prepared and diluted at appropriate concentrations (10^{-4} and $10^{-1} \text{ mol L}^{-1}$) in order to determine the calibration curve. A TISAB solution (mixture of aqueous solutions at 0.1 mol L^{-1} of KNO_3 , $\text{C}_2\text{H}_4\text{O}_2$ and $\text{C}_2\text{H}_3\text{NaO}_2$) was prepared and added in all standard solutions and samples to maintain the ionic strength during the measurements. The partition coefficient of each IL, K_{IL} , was determined according to Eq. (2).

$$K_{IL} = \frac{C_{ILT}}{C_{ILB}} \quad (2)$$

where C_{ILT} and C_{ILB} are the concentrations (g L^{-1}) of the ionic liquid in the top and in the bottom phase, respectively. The top phase corresponds to the PEG-rich phase in the systems using ILs as adjuvants.

3. Results and discussion

3.1. Effect of the ABS tie-line length in the biomolecules partition coefficients

The partition coefficients of the biomolecules were initially determined in the various ABS under study at a constant mixture composition: 30 wt% of PEG + 30 wt% of salt; 30 wt% of IL + 30 wt% of salt; and 30 wt% of PEG + 30 wt% of salt + 5 wt% of IL. The first two will be referred thereafter as ternary systems, while the last one comprising the use of ILs as adjuvants as quaternary systems. This fixed mixture composition allows the evaluation of the effect of the tie-line length (TLL, which represents the difference in compositions between the coexisting phases) in the partition coefficients. The detailed partition coefficients and respective standard deviations of the several biomolecules at this mixture composition of the ternary and quaternary ABS are reported in the Supporting Information (Tables S3–S8).

The results obtained for the partition coefficients for phenolic antioxidants, amino acids and alkaloids, as a function of the TLL,

are depicted in Fig. 2. In all systems, and as a result of the citrate salt salting-out effect, the biomolecules preferentially partition to the polymer-rich phase in polymer-salt and polymer-salt-IL systems, and to the IL-rich phase in IL-salt ABS. Curiously there is not a common trend for the different types of biomolecules investigated since for phenolic compounds the partition coefficients decrease with the increase of the TLL, while the opposite behavior is observed for amino acids; for alkaloids there is no significant effect of the TLL on the partition coefficients. Even so, general trends are observed for each family of biomolecules that seem to be independent of the ABS type. According to the K_{ow} values given in Table 1, phenolic compounds are amongst the most hydrophilic compounds investigated and, as a result, the magnitude of the K values decreases with the increase of the TLL. With the increasing difference in the phases compositions as the TLL increases, the PEG-rich phase becomes more hydrophobic, thus reducing the partition of phenolic compounds to this phase. The opposite behavior is observed for amino acids, the most hydrophobic compounds investigated, which have an enhanced partition to the PEG-rich as the TLL increases and as this phase becomes more hydrophobic. Finally, no significant dependence is observed for alkaloids, which in terms of hydrophobicity given by the octanol-water partition coefficients fall between the phenolic compounds and amino acids.

Given the influence of the TLL on the partition coefficients, further partitioning studies were conducted at a fixed TLL (*ca.* 70) aiming at isolating the effect of ILs as adjuvants in polymer-salt ABS from the TLL effect, as discussed below.

3.2. Effect of the ABS type in the biomolecules partition coefficients

The partition of biomolecules in ABS results from salting-out effects and specific interactions that occur between the target solute and the phase-forming components, while their magnitude may further depend on the phases compositions [18,26]. The nature of these interactions in PEG + salt ABS and how these are affected by the presence of ILs is the goal of this study. Therefore, the partition coefficients of all biomolecules in the different ABS types were determined at a fixed TLL (*ca.* 70). For all systems and biomolecules investigated the partition coefficient (K) values are higher than 1.0, showing the biomolecules preferential partition to the most hydrophobic phase (polymer-rich or IL-rich phase), a trend that is also a result of the salting-out effect induced by the salt.

The partition coefficients of the phenolic antioxidants in the various ABS studied at a fixed TLL are reported in Fig. 3. The results for the PEG + salt ABS are represented by the continuous line, with the dashed lines corresponding to the uncertainty on that value, allowing for a direct comparison with the results obtained for the IL + salt and PEG + salt + IL ABS.

The partition coefficients of antioxidants in ABS composed of PEG + salt or PEG + salt using ILs as adjuvants are higher than those observed in the IL + salt systems (without PEG). The polymer-based systems display thus a better performance to extract phenolic compounds than those composed of just ILs. At the pH used in these studies the phenolic compounds are negatively charged and still prefer the PEG-rich phase with a lower ionic strength, meaning that electrostatic interactions do not play a dominant role in the partition. There are no significant differences between the various ILs studied in the extraction of the phenolic antioxidant with exception of the ammonium and phosphonium IL + salt ABS used for the extraction of gallic acid. However, even in these cases there seems to be no correlation between the performance of the IL + salt ABS and the use of the same IL as adjuvant in PEG + salt ABS. This suggests that the extraction is not driven by specific interactions between the IL and the solute but mainly by the differences in hydrophobicity between the phases.

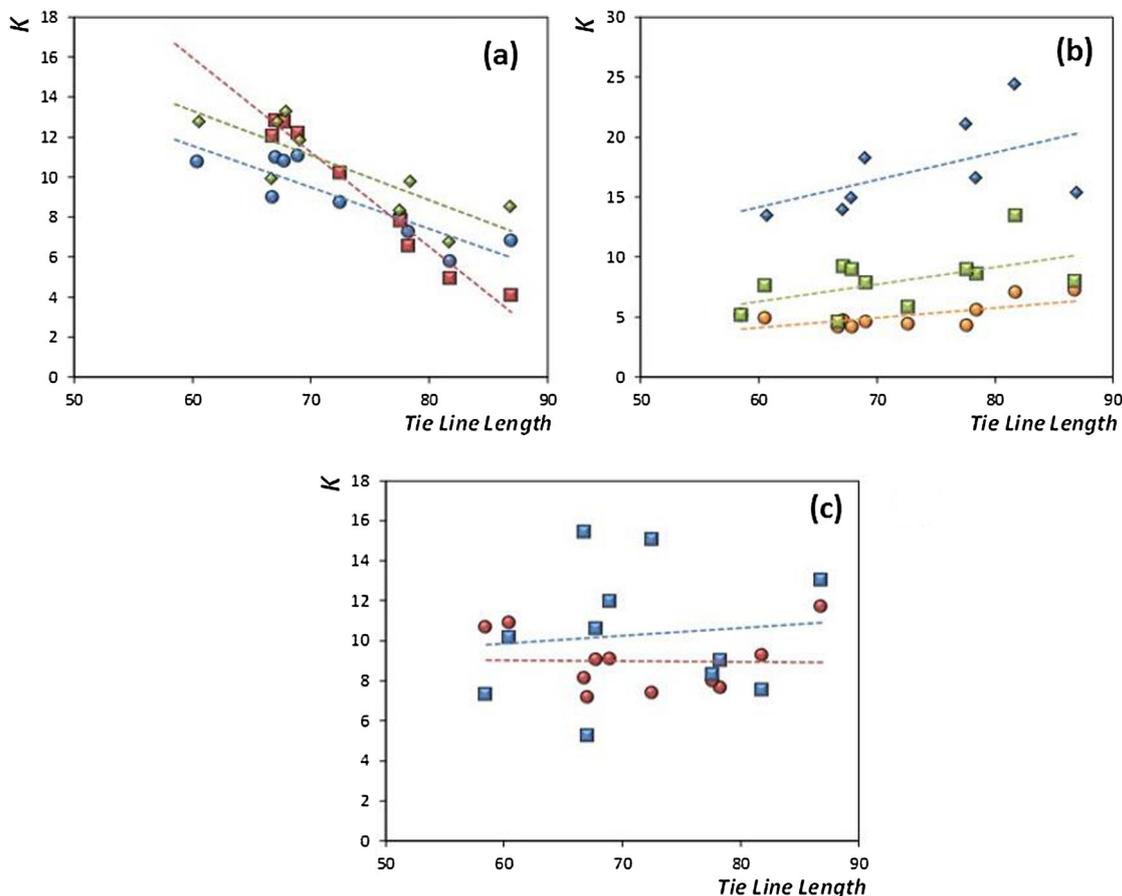


Fig. 2. Effect of TLLs on the partition coefficients of several biomolecules in ternary and quaternary ABS: (a) antioxidants - vanillic acid (●), eugenol (◆), gallic acid (■), (b) amino acids - L-Tryptophan (◆), L-Phenylalanine (■), L-Tyrosine (●); and (c) alkaloids - nicotine (●), caffeine (■). Dashed lines (–) correspond to linear regressions applied to the experimental data.

The results of partition coefficients here obtained show a positive effect when using ILs as adjuvants for the compounds of lower hydrophobicity (gallic acid ($\log K_{ow} = 0.7$) and vanillic acid ($\log K_{ow} = 1.33$)), but for eugenol ($\log K_{ow} = 2.99$) the opposite effect is observed. It seems thus that the performance of ILs as adjuvants is dependent on the hydrophobicity of the biomolecule. For the vanillic acid the increase observed in the K values in quaternary ABS is similar to that previously reported for the partition of antioxidants in ABS formed by PEG 300 + Na_2SO_4 + 5 wt% IL [22].

The partition coefficients of amino acids at 298 K in the PEG + Salt, IL + Salt or PEG + Salt + IL systems studied, at a common TLL (ca. 70), are shown in Fig. 4.

Zaslavsky et al. [27] studied the partition of amino acids in PEG-based ABS and demonstrated that the hydrophobic effect is the most relevant factor for this type of separation. As can be seen in Fig. 4, the partition coefficients increase with the increase of the amino acids $\log K_{ow}$ (–1.06 for tryptophan, –1.38 for phenylalanine, and –2.26 for tyrosine), in agreement with the conclusions of Zaslavsky et al. [27].

Pereira et al. [18] demonstrated that the incorporation of 5 wt % of an IL in a PEG 600 + Na_2SO_4 ABS improves the extraction capacity of L-tryptophan to the PEG-rich phase. A similar, albeit weaker, improvement is here observed for both tryptophan and phenylalanine, but not for tyrosine. This amino acid, the most hydrophilic compound here used, shows a unique behavior amongst all the biomolecules studied, with partition coefficients higher in IL + salt ABS than in any of the other ABS studied. This suggests that in IL + salt ABS the partition may be dominated by specific interactions with the IL, with different cations displaying

significant differences in the partition coefficients. Given the trend obtained for the ILs, and based on the charged nature of tyrosine at pH 7, it seems that the major interactions occur with the IL chloride anion, both by electrostatic and hydrogen-bonding interactions (ILs with weaker cation-anion cohesive energies, such as $[\text{P}_{4444}]\text{Cl}$, will allow stronger interactions of Cl^- with the amino acid solute) [28]. Moreover, and based on the ILs rank in IL + salt ABS to extract tyrosine, the ILs hydrotropic phenomenon may also be playing a role [29]. In summary, these specific amino acid-IL interactions are particularly relevant for solutes of lower hydrophobicity. On the other hand, the partition of the remaining amino acids in PEG + salt ABS is probably dominated by differences in hydrophobicity between the phases, as discussed below, and the use of 5 wt% of ILs as adjuvants has a minor impact upon their partition. That the partition coefficients for L-phenylalanine are similar in both PEG + salt, and on the systems with adjuvant, and that no differences are observed for the various ILs supports this idea.

The partition coefficients of the alkaloids nicotine and caffeine in the ABS studied are shown in Fig. 5.

The K values of the alkaloids are >1.0 , showing the biomolecules preferential partition for the most hydrophobic phase in all systems investigated (IL-rich phase in IL + salt ABS and PEG-rich phase in PEG + salt and PEG + salt + IL ABS). As observed before for most biomolecules, the IL + salt systems performance for the partition of alkaloids is worse than that shown by the polymer-based ABS (both for ternary and quaternary systems). However, for the studied alkaloids, the presence of ILs as adjuvants induces a significant increase in the partition coefficients.

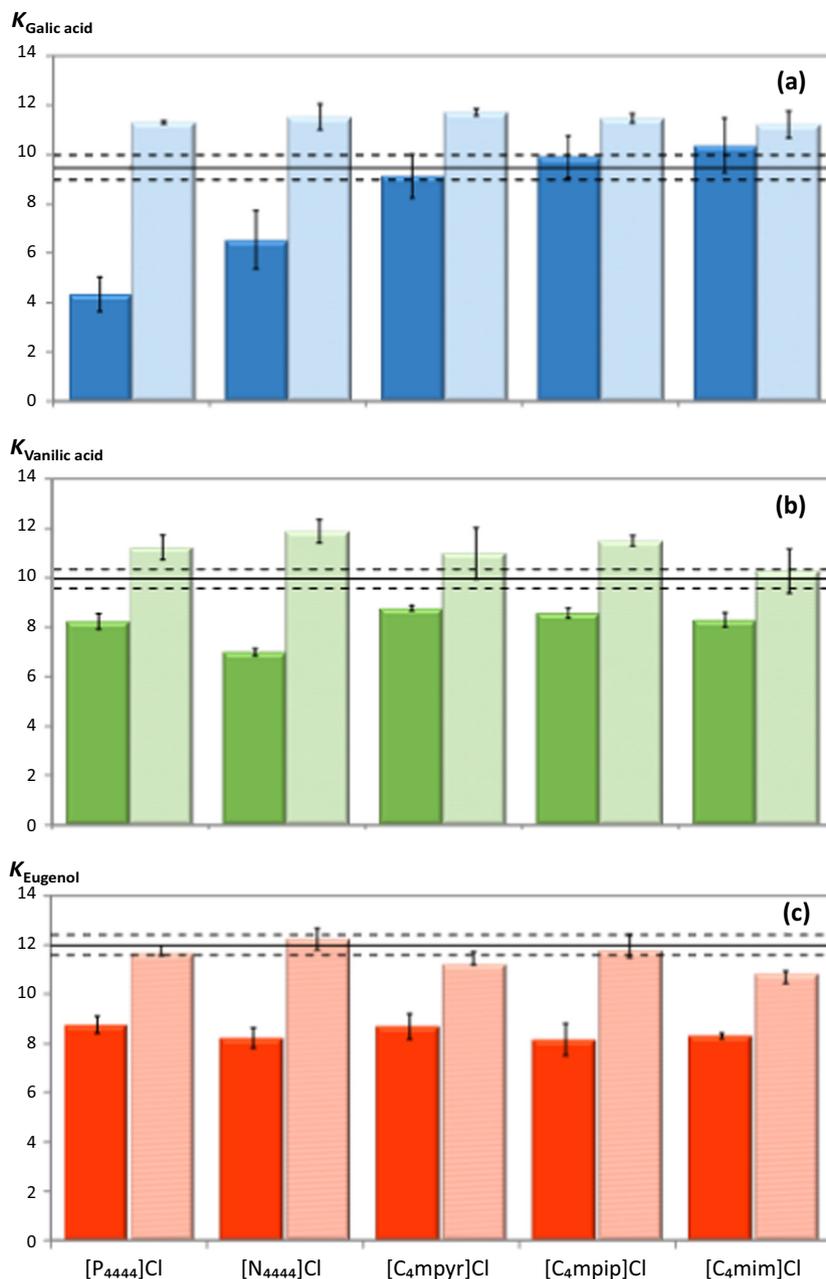


Fig. 3. Partition coefficients (K) of (a) gallic acid, (b) vanillic acid and (c) eugenol, at 298 K and at a fixed TLL, for the systems composed of PEG 400 + citrate buffer pH 7.0 (-); of IL + citrate buffer (dark blue, green and red) and of PEG 400 + citrate buffer + 5 wt% IL K (light blue, green and red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In summary, and based on the overall gathered results, it seems that IL + salt ABS perform better in the extraction of more hydrophilic compounds (particularly seen with ι -tyrosine, $\log K_{ow} = -2.26$), in which specific interactions with the IL play a significant role, PEG + salt systems display higher extraction abilities for solutes of more extreme hydrophobicity (such as eugenol, $\log K_{ow} = 2.49$), supporting the relevance of having phases of higher hydrophobicity such as the PEG-phase, and PEG + salt + IL (as adjuvants) systems are of higher performance in the extraction of hydrophobic solutes (such as vanillic acid, gallic acid, caffeine, nicotine and eugenol, with $\log K_{ow}$ values ranging between -0.07 and 2.49), in which the differences in hydrophobicity between the phases seem to dominate the partition trend, as discussed in more detail below.

3.3. Effect of the IL partition in the biomolecule partitioning

Fig. 6(a–c) depicts the partition coefficient of all biomolecules as a function of the IL coefficient partition in the studied quaternary systems, at the fixed TLL of 70. The detailed partition coefficients of ILs and respective standard are reported in the Supporting Information (Table S9). The ILs investigated preferentially partition to the PEG-rich phase, with partition coefficients ranging between 4 and 6. The preferential IL partition to this phase changes its properties, in particular the hydrophobicity of the phase, modifying thus the extraction ability of systems comprising ILs as adjuvants.

It can be seen in Fig. 6(a–c) that there is a correlation of the K of the biomolecules and K_{IL} , meaning that the presence of the IL at the

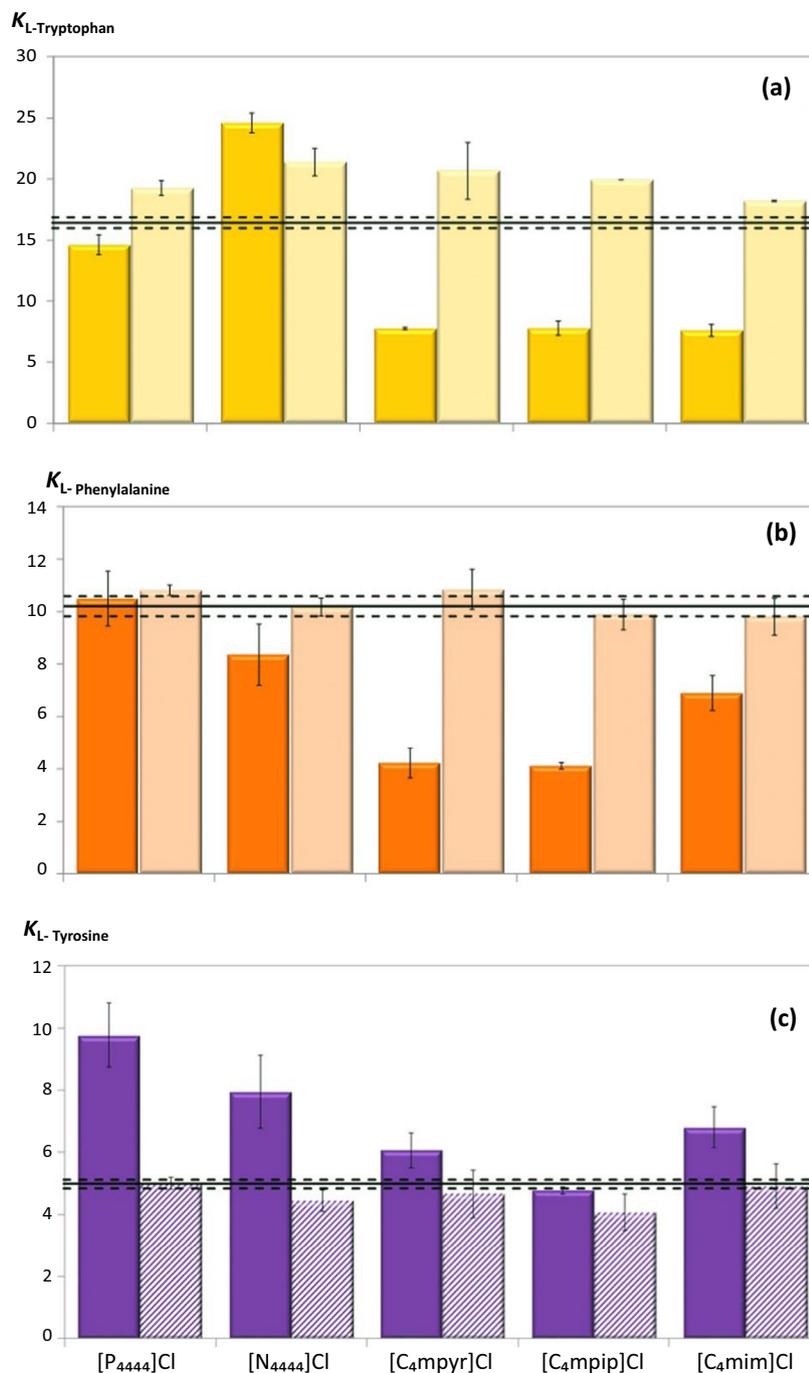


Fig. 4. Partition coefficients (K) of (a) L -Tryptophan, (b) L -Phenylalanine and (c) L -Tyrosine, at 298 K and at a fixed TLL, for the systems composed of PEG 400 + citrate buffer pH 7.0 (–); of IL + citrate buffer (dark yellow, orange and violet) and of PEG 400 + citrate buffer + 5 wt% IL (light yellow, orange and violet). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

PEG-rich phase enhances the partitioning of these biomolecules. This increase is further proportional to the log K_{ow} of the biomolecules (Fig. 6(d)). It should be however highlighted that two outliers have been found and removed from the correlation, corresponding to L -Tryptophan and nicotine. Even so, and given the large number of solutes investigated, it is clear that the presence of IL in the PEG-rich phase enhances the extraction of more hydrophobic biomolecules, while the opposite behavior is observed for L -Tyrosine, the most hydrophilic compound investigated. These results support the idea of a partition driven by the hydrophobicity difference between the two phases, as previously discussed.

The large amount of results obtained in this work allow to draw the following conclusions:

- (i) In general, with exception of the tyrosine, the performance of the IL + salt ABS for the extraction of the biomolecules studied is worse than that displayed by PEG + salt ABS.
- (ii) There is in general a favourable effect of the use of ILs as adjuvants in PEG + salt ABS, particularly seen for more hydrophobic compounds (with the exception of eugenol, the most hydrophobic biomolecule studied and for which there is already a high partition coefficient in the PEG + salt ABS).

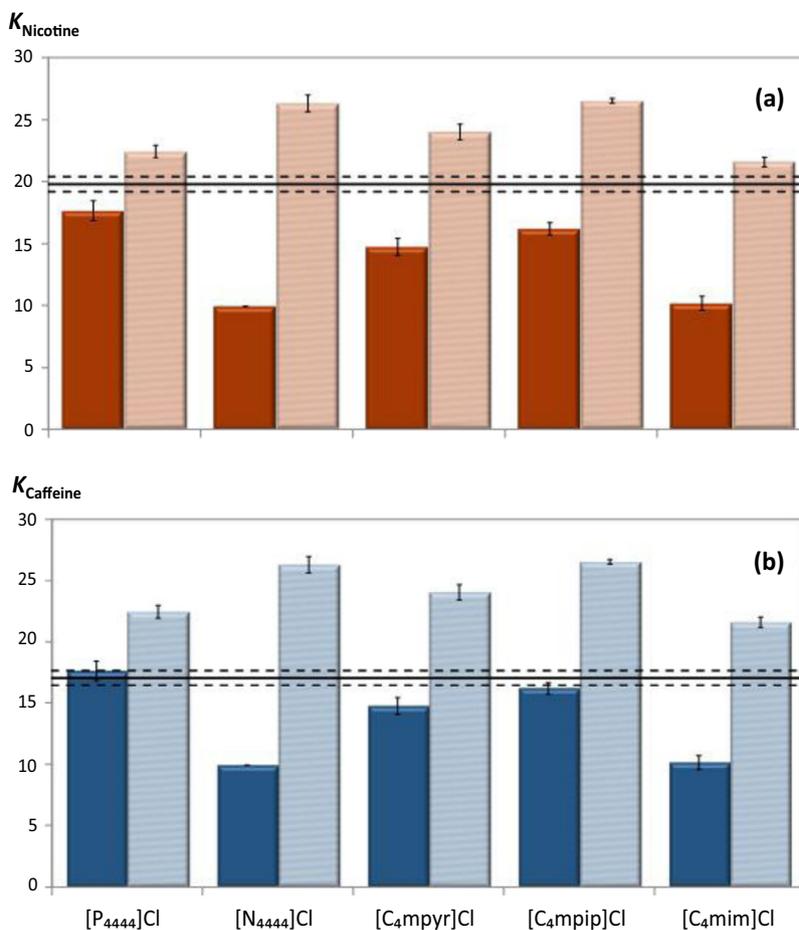


Fig. 5. Partition coefficients (K) of (a) nicotine and (b) caffeine, at 298 K and at a fixed TLL, for the systems composed of PEG 400 + citrate buffer pH 7.0 (-); of IL + citrate buffer (dark brown and blue) and of PEG 400 + citrate buffer + 5 wt% IL (light brown and blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

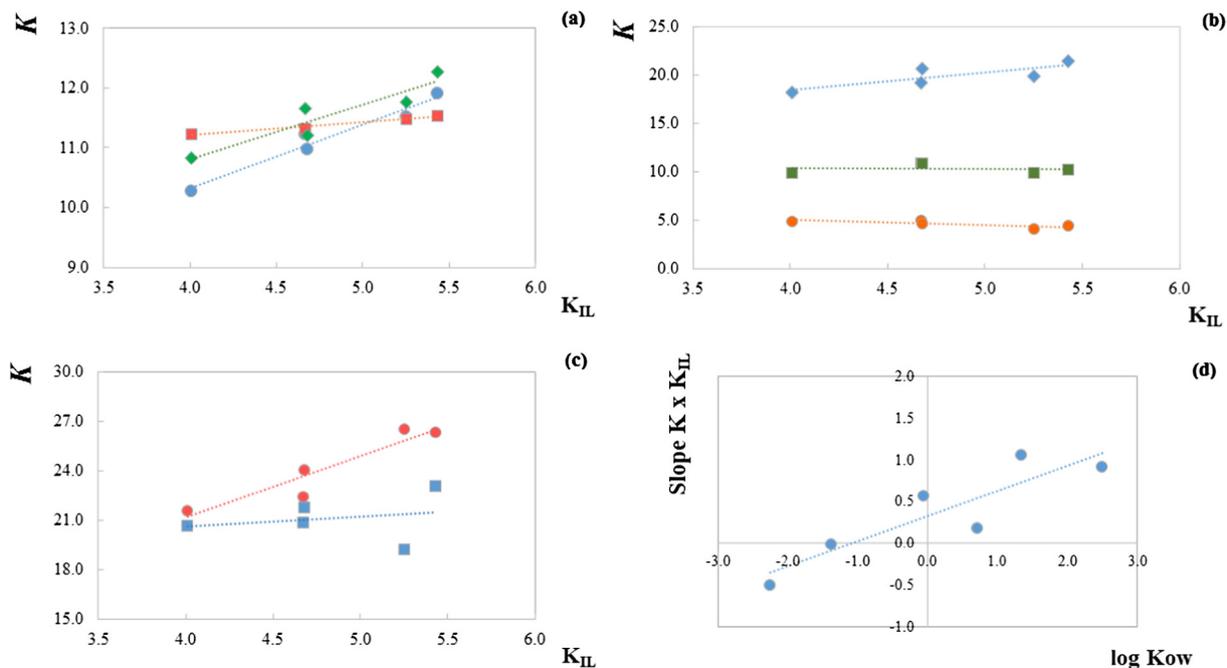


Fig. 6. Effect of K_{IL} on the partition coefficient of the studied biomolecules in the quaternary ABS at a fixed TLL: (a) antioxidants - vanillic acid (●), eugenol (◆), gallic acid (■), (b) amino acids - L-Tryptophan (◆), L-Phenylalanine (■), L-Tyrosine (●); and (c) alkaloids - nicotine (●), caffeine (■). (d) Slope of the curve of K versus K_{IL} as a function of $\log K_{ow}$. Dotted line (–) corresponds to linear regression.

- (iii) The favourable partition of more hydrophilic biomolecules in IL + salt ABS seems to be ruled by specific interactions with the IL, while the favourable partition of more hydrophobic biomolecules in PEG + salt and PEG + salt + IL seems to be governed by the differences in the phases hydrophobicities.

When dealing with PEG + salt ABS using ILs as adjuvants the effect obtained seems to be dependent on the hydrophobicity of the biomolecule and hydrophobicity of the phases rather than on its ability to establish specific interactions with the ILs. The strong salting-out nature of the citrate salt may be masking or preventing the establishment of these specific interactions, and this may be another aspect that may explain the conflicting results reported in the literature for the effect of ILs as additives in PEG + salt ABS, since most studies were carried out with strong salting-out inducing salts [18,22]. The nature of the salt will be object of a future work aimed at elucidating its role on the use of ILs as additives on polymer + salt ABS.

4. Conclusions

The use of ILs as adjuvants in conventional PEG + salt ABS has been proposed in the literature to enhance the extraction of biomolecules. Previous works reported conflicting results, and to shed light on the behavior of these systems, the effect of ILs used as adjuvants in small concentrations (5 wt%) in ABS composed of PEG 400 and potassium citrate buffer at pH 7.0 was here investigated, through the study of the partition coefficients of a large number of biomolecules of different nature and covering a wide range of hydrophobicities.

In the first section of this work, the influence of the TLL in the partition of the biomolecules was studied. It was observed that for the phenolic antioxidants, the most hydrophilic compounds investigated, the partition coefficients decrease with increasing TLL. The opposite behavior was observed for the amino acids, which comprise some of the more hydrophobic compounds studied, and no significant dependence on the partition coefficients with the TLL was identified for the alkaloids, which in terms of hydrophobicity given by the octanol-water partition coefficients are placed between phenolic compounds and amino acids.

In the second section of the work, a constant TLL was adopted to study the effect of ILs as adjuvants in the partition of the biomolecules, compared against the extraction performance of IL + salt and PEG + salt ABS. The obtained results show that the use of a small concentration of IL in quaternary systems has a favourable effect on the partition coefficients of most of the biomolecules studied, when compared with PEG + salt and IL + salt ABS, the only exceptions being eugenol and tyrosine, respectively the most hydrophobic and hydrophilic biomolecules studied. Eugenol is the most hydrophobic compounds studied, with high partition coefficients already observed in the PEG + salt ABS. On the other hand, IL + salt ABS display a better performance to extract more hydrophilic solutes, such as tyrosine, in which the effect of the IL nature is also more relevant, supporting therefore IL-solute specific interactions occurring particularly in these type of systems and type of biomolecules. In relation to the antioxidants, for gallic acid and vanillic acid, partition coefficients increased by 20.4% and 15.5% in quaternary systems in relation to ABS without IL. With respect to alkaloids, nicotine and caffeine partition coefficients increased by 24.5% and 20.8%, respectively, in quaternary systems compared to ternary systems (without IL). In these studies, no significant differences between the various ILs were identified. This suggests that the hydrophobicity of the biomolecule being extracted and the hydrophobicity of the phase afforded by the presence of IL play

the dominant role on controlling the partitioning of hydrophobic biomolecules in quaternary systems, and that no significant specific interactions are induced by the ILs on these systems. These conclusions are supported by a linear trend found between the partition coefficients of the IL and biomolecule to the PEG-rich phase and the octanol-water partition coefficients of the studied biomolecules. It should be however highlighted that the absence of specific interactions in this type of systems may be due to the use of a strong salting-out salt.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.seppur.2017.07.018>.

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